The impact of commercially available purinergic ligands on purinergic signalling research

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Abstract Due to the extremely wide-spread expression of purinergic receptors, purinergic signalling has been implicated in numerous physiological and pathophysiological areas. To better understand the involvement of purinergic receptors in such areas, the researcher's requirement for diverse and varied purinergic receptor ligands has greatly increased. This has generated increased commercial opportunities for life science suppliers, and ultimately, has led to a rapid expansion in the number of commercially available purinergic receptor ligands. The wide-spread availability of ligands to researchers has greatly benefited the scientific community, nurturing the rapid and continued expansion of the purinergic signalling field.

Keywords Purinergic receptor · Adenosine receptor · Ligands · Glutamate · Commercial development

Introduction

The identification and latter acceptance that adenosine-5'-triphosphate (ATP) is fundamentally involved in extracel-

lular signalling as a co-transmitter in all nerves, in both the central and peripheral nervous systems led to the foundation of the purinergic signalling field [1]. The further discovery and subsequent cloning of the purinergic receptors prompted the establishment of the P1 (adenosine) and P2 receptor groups of which the P1 receptor group is subcategorised into the A₁, A_{2A}, A_{2B} and A₃ receptors and the P2 receptor group into the P2X subcategory, composed of the P2X_{1–7} receptors and the P2Y subcategory composed of P2Y_{1, 2, 4, 6, 11–14} receptors [1].

The wide-spread expression of these purinergic receptors, in addition to the diverse and abundant role of these receptors in extracellular signalling has led to the recognition of both short-term purinergic signalling in neurotransmission, neuro-modulation, and neurosecretion and long-term (trophic) signalling in controlling cell proliferation, differentiation, motility, death and regeneration [2]. Moreover, the extensive implication of purinergic signalling in such physiological and pathophysiological areas has led to a great deal of interest in the characterisation of the numerous roles and functions of the purinergic receptors.

Ascent Scientific (see Box 1), a life sciences supplier with a core ethos of helping and progressing research by providing high quality biochemicals of exceptional value to the research community, has recently begun an expansion of their purinergic receptor ligand range in order to meet the ever increasing demands of researchers for purinergic receptor ligands. By endeavouring to meet this demand,

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Ascent Scientific hope to enable researchers and institutes to further progress the rapidly expanding purinergic research field.

Ascent Scientific company profile and history

Steve Roome PhD and Iain Sandison were two research chemists, who worked closely with pharmacologists, physiologists and life scientists. “Our friends kept telling us how hard it was to get research grants, and how research was being hampered by inconsistent quality, and high costs of reagents and small molecule tools. We knew we had the skills to help, so we decided to do something about it. Ascent Scientific was born out of a desire to offer a better solution to scientists – excellent value, without of course, compromising quality.

So in 2005 the two friends opened a small laboratory in the south of England, UK, and started offering a custom synthesis service to their friends and colleagues. “Keeping costs low, yet maintaining quality was our main priority – after investing in top of the range QC and Analytical equipment, we built our first lab using glassware purchased from E-Bay and IKEA kitchen parts! Things have moved on since then, but our commitment to offering exceptional value and high quality has continued.”

Following those early beginnings in 2005, Ascent Scientific, now an Abcam company, offers a range of biochemicals to research scientists across the world. Ascent Scientific’s mission is to progress research by providing biochemicals of high purity and exceptional quality, and their products have been cited in numerous peer-reviewed publications. The high purity range includes agonists, antagonists, ion channel modulators, enzyme inhibitors and signalling tools.

“My research examines the regulatory mechanisms controlling GPCR traffic whilst assessing the impact of such mechanisms on signalling pathways that initiate important physiological responses. As researchers, we need a reliable source of high purity tools – from adenosine and purine agonists and antagonists, through to modulators of downstream signalling pathways. Companies like Ascent Scientific offer high purity reagents at exceptional value – allowing us to carry out even more valuable research in this complex field.”

Stuart Mundell PhD, Lecturer / BHF Research Fellow
University of Bristol

“Purinergic and adenosine ligands are often complex molecules from a synthetic point of view. Manufacture of these highly polar & water soluble molecules can be lengthy and purification is often very challenging. The target ligand is often based upon a nucleotide with the final step being the addition of a polyphosphate unit to a nucleoside. This chemistry is difficult to do cleanly, resulting in complex mixtures of very similar products that need to be separated and characterised. Minor impurities can cause a major effect in biological experiments, so it is imperative that the target is clean and proven to be clean with high quality analytical data.”

Steve Roome PhD
General Manager,
Ascent Scientific

Box 1 – the company profile and history of Ascent Scientific.

In order to satiate the ever-growing appetite of researchers for purinergic receptor ligands, the development and proceeding commercial development of such ligands by companies

such as Ascent Scientific has greatly benefited the scientific community, allowing the ample provision of purinergic receptor ligands to researchers. This wide-spread availability

has allowed and fostered the dramatic growth in the purinergic research signalling field, in which a great number of key ligands have widely been utilised by the scientific community to continue the characterisation of the numerous and diverse roles and functions of the P1 and P2 receptors.

This article introduces a number of these key P1 and P2 receptor ligands (Tables 1 and 2) which have been commercially developed and made available by companies such as Ascent Scientific and briefly describes how they have led to notable advances in the purinergic research field.

P2 receptors

Asc-009—PPADS

The non-specific, potent P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2',5'-disulfonic acid (PPADS) has been readily and commercially available for some time and blocks the P2X₁, P2X₂, P2X₃, P2X₅ and P2X₄ receptors (IC₅₀=1, 2, 1, 27.5, 3, 4.2 nM at P2X₁, 2, 3, 4, 5, 7, respectively and 6 and 15 nM at the P2Y₁, 4 receptors, respectively [3, 30, 31]). Although a standard tool, PPADS has played a significant role in unravelling purinergic physiology and signalling. Notably, by blocking the P2X₄

receptor with PPADS and activating it with TNP-ATP, Inoue et al. [31] recently showed that marked tactile allodynia depends upon the P2X₄ receptor in the spinal cord. Additionally, by blocking P2X₃ and P2X_{2/3} receptors, PPADS has also been shown to reduce micturition reflex contraction in mice revealing that both presynaptic P2X₃ and P2X_{2/3} receptors are involved in the facilitation of the micturition reflex contraction [32].

In addition to ATP acting as a co-transmitter, the extracellular nucleotide and product of ATP dephosphorylation, adenosine diphosphate (ADP) has also been shown to display activity at P2 receptors. ADP, much like ATP acts at various purinergic receptors and has been implicated in a number of research areas. For instance by acting at P2Y₁₃ receptors, ADP has been shown to mediate insulin secretion [33]. Interestingly, ADP has also been fundamentally associated with platelet research and is known to play a role in platelet activation. Furthermore, ADP has additionally been shown to be stored in platelets and is also released following platelet activation [34]. Thus, certain ADP-sensitive purinergic receptors, notably including the P2Y₁ and P2Y₁₂ receptors, have been shown to be essential in a number of platelet functions including platelet shape change and platelet activation [34, 35].

Recent commercial development of purinergic receptor ligands and the utilisation of such ligands has allowed an

Table 1 Table showing key P2 receptor ligands available from Ascent Scientific and the potency of these ligands at respective P2 receptors

Key P2 receptor ligands				
Ligand name	Ligand Type	IC ₅₀ value (nM) unless stated otherwise		References
PPADS	Antagonist	P2X ₁ =1 P2X ₃ =1 P2X ₅ =3 P2Y ₁ =6	P2X ₂ =2 P2X ₄ =27.5 P2X ₇ =4.2 P2Y ₄ =15	[3]
UDP sodium salt	Agonist	P2Y ₆ =30	P2Y ₁₄ =350 (EC ₅₀)	[4, 5]
UDP-glucose	Agonist	P2Y ₁₄ =82 (EC ₅₀)		[6]
	Agonist	P2X ₁ =0.60 (EC ₅₀) P2X ₄ =9.1 (EC ₅₀)	P2X ₃ =1.3 (EC ₅₀) P2X ₆ =0.5 (EC ₅₀)	[7, 8]
(+/-)-Clopidogrel	Antagonist	—		—
Brilliant Blue G	Antagonist	hP2X ₇ =200	P2X ₄ =3.2 μM	[9]
β-Nicotinamide adenine dinucleotide	Agonist	P2Y ₁ =6.1 (EC ₅₀)		[10]
A 438079	Antagonist	P2X ₇ =6.5 (pIC ₅₀)		[11]
MRS 2179	Antagonist	P2X ₁ =1.15 P2Y ₁ =0.33	P2X ₃ =12.9	[12]
NF449	Antagonist	P2X ₁ =0.05	P2X ₇ =40 μM	[13]
KN-62	Antagonist	P2X ₇ =25		[14]
Suramin	Inhibitor	—		
MRS 2578	Antagonist	hP2Y ₆ =37		[15]
Bz-ATP	Agonist	P2X ₇ =15 μM (EC ₅₀)		[8]
MRS 2211	Antagonist	P2Y ₁₃ =pIC ₅₀ =5.97		[16]
NF023	Antagonist	P2X ₁ =0.34 μM	P2X ₃ =8.5 μM	[17]
NF157	Antagonist	P2X ₁₁ =0.5 μM		[18]

increased understanding of the role of ADP, ATP and the purinergic receptors in platelet function and indeed further research into this area may prove promising in the future to develop potential thrombotic treatments.

Asc-387—clopidogrel

Clopidogrel, a potent antagonist selective for the P2Y₁₂ receptor, inhibits ADP-induced platelet aggregation and activation and as such has been extremely useful in characterising anti-thrombotic action. The thienopyridine active metabolite of clopidogrel specifically and irreversibly inhibits the P2Y₁₂ receptor found on platelets, leading to inhibition of platelet fibrinogen binding and allowing the consequent anti-thrombotic action of clopidogrel [36, 37]. Kanko et al. [38] revealed that clopidogrel also maintains a reducing effect on tissue nitric oxide (NO) levels suggesting that clopidogrel may additionally show other potential therapeutic uses in addition to displaying anti-thrombotic action.

Asc-451—dipyridamole

Like clopidogrel, dipyridamole also decreases platelet aggregation. This non-selective phosphodiesterase inhibitor inhibits degradation of cyclic AMP and cyclic GMP (IC₅₀=5 and 3 µM, respectively) [39]. Dipyridamole prevents thrombus formation by inhibiting the cellular uptake and degradation of adenosine by functioning as a nucleoside transport inhibitor [40]. Moreover, dipyridamole has also been particularly useful in characterising P1 receptor mechanisms by allowing higher extracellular adenosine levels [41].

Asc-414—MRS 2179

A potent and selective P2Y₁ receptor antagonist (IC₅₀=0.33 nM) [12], MRS 2179 has been shown to inhibit ADP-induced platelet shape change aggregation [42]. In addition to being active in vivo, MRS 2179 has shown promise as an anti-thrombotic therapy and has implicated P2Y₁ as a promising potential target for thrombotic syndromes [43]. Furthermore, by potently antagonising P2Y₁ receptors, MRS2179 has been shown to inhibit ATP-induced release of glutamate in astrocytes and further to this, has been utilised to inhibit relaxation of longitudinal and circular muscle [44, 45].

Asc-415—NF449

Similarly to MRS2179, NF449 is a potent antagonist which is highly selective for P2X₁ receptors (IC₅₀=0.05 nM) and as with many other P2Y₁ receptor ligands, NF449 has been utilised in platelet function research [13]. By selectively blocking the P2X₁ receptor, NF449 has been shown to reduce collagen-induced platelet aggregation and inhibit calcium influx [46]. This in turn has confirmed the role of P2X₁ receptors in platelet activation [13]. Due to the selectivity NF449 shows at P2X₁, NF449 may itself also represent potential as an anti-thrombotic drug [46].

Asc-389—Brilliant Blue G

Brilliant blue G, the selective, non-competitive P2X₇ and P2X₄ receptor antagonist (IC₅₀=200 nM at hP2X₇ receptors

Table 2 Table showing key P1 ligands available from Ascent Scientific and the potency of these ligands at respective P1 receptors

Key P1 receptor ligands						
Ligand name	Ligand type	K _i values (in nM) unless stated otherwise				References
		A ₁	A _{2A}	A _{2B}	A ₃	
2-Chloroadenosine	Agonist	300	80		1,900	[19]
ZM 241385	Antagonist	255	1	50	>19 µM	[20]
Caffeine	Antagonist	50 µM	30 µM			[21]
DPCPX	Antagonist	3.9	130	50	4,000	[22]
MRS 1754	Antagonist	403	503	1.97	570	[22]
N ⁶ -Cyclopentyl-adenosine	Agonist	2.3	790		43	[23]
2-Chloro-N ⁶ -cyclopentyl-adenosine	Agonist	0.8	2,300		42	[24]
IB-MECA	Agonist	12.6	56		1.1	[25]
SCH 58261	Antagonist	289	1-2		>10,000	[21]
NECA	Agonist	14	20	330	6.2	[26]
8-(3-Chlorostyryl)caffeine	Antagonist		54			[27]
Iodotubercidin	Antagonist	26 at Adenosine Kinase				[28]
CGS21680	Agonist	23				[29]

and 3.2 μM at P2X_4 [10]), has allowed the study of P2X_7 and P2X_4 receptors in a variety of research areas and by selectively and non-competitively blocking the P2X_7 receptor has asserted the importance of the P2X_7 receptor in processes such as cytokine release and pain [47]. More recently, Brilliant Blue G has been used to show that P2 receptors play a vital role in the entry of the Hepatitis B and D viruses [48].

Asc-413—A 438079

A 438079 is a competitive, potent P2X_7 receptor antagonist (pIC_{50} value=6.5 nM) [11] which, similar to Brilliant Blue G, has been implicated in pain both *in vitro* and *in vivo* and has been shown to reduce pathological nociception [49, 50]. Further to this, A 438079 has been used in a number of studies that have investigated P2X_7 receptor antagonism as a potential therapeutic strategy in both pain and neurological disorders, such as Parkinson's disease [51]. For instance, A 438079 has been shown to significantly prevent the depletion of striatal dopamine stores, suggesting that blockade of P2X_7 receptors may represent a novel protective strategy for striatal dopamine terminals in Parkinson's disease [51].

Asc-421—KN-62

In addition to having been implicated in areas such as pain and neurological diseases, the P2X_7 receptor has also been shown to be essential in other physiological areas and has notably been associated with cell proliferation and apoptosis [52]. KN-62, in addition to being a reversible and selective inhibitor of Cam Kinase II, is a potent, non-competitive antagonist at the P2X_7 receptor (IC_{50} value=25 nM) [14]. There are currently few P2X_7 receptor blockers, however, KN-62 is considered to be one of the most potent. By blocking the P2X_7 receptor KN-62 inhibits reduction in cell viability by preventing a bidirectional flux of cations which triggers depolarisation [52]. This has revealed that the P2X_7 receptor is essential to cell cytotoxicity and crucially, has shown that P2X_7 receptor activation can both stimulate cell proliferation and cause cell death [52].

Asc-383—UDP sodium salt

Uridine diphosphate (UDP), similar to ATP and ADP, is an endogenous nucleotide. UDP displays activity at both the P2Y_6 and P2Y_{14} receptors (IC_{50} =30 nM at P2Y_6 , EC_{50} =350 nM at P2Y_{14} [4, 5]). The P2Y_6 receptor is expressed on microglia and it is thought that by activating this receptor, UDP mediates microglial phagocytosis [53]. Use of UDP has also helped to clarify the role of the previously

orphaned P2Y_{14} receptor, which has since been implicated in immune and inflammatory cells [4, 54, 55].

Asc-384—UDP-glucose

Similar to UDP, the synthesis and commercial availability of UDP-glucose, a highly potent endogenous agonist of the P2Y_{14} receptor (EC_{50} =82 nM) [6], has allowed further characterisation of P2Y_{14} receptor functionality, and has aided the definition of the as yet unclear immunological role of the P2Y_{14} receptor [4]. In addition to an immunological role, recent studies with UDP-glucose have also shown that the P2Y_{14} receptor is involved in the modulation of gastric function [56].

Asc-403— β -NAD

Interestingly, it has recently been suggested that another molecule, β -nicotinamide adenine dinucleotide (β -NAD) like ATP, may also be a co-transmitter and may likely represent a novel extracellular signalling molecule [57, 58]. Smyth et al. [58] revealed that β -NAD is released from sympathetic nerve terminals and as such suggested that as with ATP, β -NAD has putative neurotransmitter or neuromodulator functions. Furthermore, β -NAD has also recently been shown to be a P2Y_1 and P2Y_{11} receptor agonist (EC_{50} =6.1 at P2Y_1) [10, 59]. The wide-spread commercial availability of this compound allows the continued characterisation of the physiological and function role of β -NAD.

P1 receptors

Adenosine, a purine nucleoside metabolite of ATP, is an endogenous ligand for P1 receptors and a neuromodulator in the nervous system. Adenosine, similar to ATP and ADP, has also been widely implicated as an important component of purinergic signalling and by acting at P1 receptors is involved in a wide variety of physiological and pathophysiological functions [60]. P1 receptor research has greatly benefited from rapid commercial development of agonists and antagonists for these receptors, allowing the continued elucidation of the four receptor subtypes (A_1 , $\text{A}_{2\text{A}}$, $\text{A}_{2\text{B}}$ and A_3) and clarification of their role and functions.

Notably, adenosine and the P1 receptors have been implicated in a number of neurodegenerative diseases. Indeed, due to adenosine's important role as a neuromodulator in the nervous system, the commercial development of P1 receptor ligands has proved highly beneficial to researchers, leading to the use of P1 receptor ligands as potential neuroprotective agents [60].

The A_1 and $\text{A}_{2\text{A}}$ receptors have received a great deal of attention in their role in such areas and both A_1 and, $\text{A}_{2\text{A}}$

receptor ligands have recently received a great deal of interest as potential drugs with neuroprotective properties.

Asc-240—caffeine

Caffeine, an A_1 and A_{2A} receptor antagonist (K_i values are 50 and 30 μM , respectively) [21] that affects cognitive function and motor behaviour, has been made widely available to researchers for many years and has been extremely useful in elucidating the role of P1 receptors [20]. Moreover, caffeine itself has been shown to display neuroprotective effects in a number of neurodegenerative diseases. For instance, by antagonising the A_1 and A_{2A} receptors caffeine reduces the negative effects of β -amyloid *in vitro* and interestingly, has been shown to prevent cognitive decline *in vitro* animal models [60–62]. Furthermore, caffeine has also been shown to be neuroprotective in Parkinson's disease models, displaying potential therapeutic effects by antagonising P1 receptors [63].

Asc-218—ZM 241385

Interestingly, ZM 241385 a potent A_{2A} receptor antagonist (K_i value=255 nM, 1 nM, 50 nM and >19 μM at A_1 , A_{2A} , A_{2B} and A_3) [19], has also been implicated in neurodegeneration and similarly to caffeine has been shown to afford neuroprotection in Alzheimer's disease models by protecting against β -amyloid neurotoxicity [60]. Furthermore in a Parkinson's disease model, ZM 241385 has also been shown to enhance L-DOPA-induced dopamine release by antagonising the A_{2A} receptor, suggesting potential therapeutic use [60].

Additionally, ZM 241385 has been essential in structural studies of the A_{2A} receptor. Jaakol et al. [64] reported the crystal structure of the A_{2A} receptor by complexing A_{2A} and ZM 241385. This remarkable breakthrough offers exciting new potential for the further understanding of A_{2A} receptors and also has implications for future GPCR drug screening and design.

Asc-439—SCH 58261

As a potent and selective A_{2A} receptor antagonist (K_i =1–2 nM), SCH 58261 has been utilised and implicated in a variety of research [21]. In addition to proving useful in the investigation of the function and mechanism of action of caffeine, SCH 58261 has also been used to block A_{2A} receptors leading to subsequent locomotor stimulation (in low doses) and cross-sensitisation [26]. This has led to the suggestion that the effect of caffeine on behavioural sensitization may be mediated through the A_{2A} receptor and has also suggested that SCH58261 may modulate dopaminergic neurons. Again, by antagonising the A_{2A} receptor, in a similar

way to caffeine and ZM 241385, SCH58261 may show potential therapeutic use in Parkinson's disease models [26].

Due to a lack of knowledge about the structural requirements for selective and potent activation of the A_{2B} receptor, and the general low affinity shown towards prototypic ligands, A_{2B} receptors, unlike other P1 receptors, are perhaps the least extensively characterised [65]. However, following an initial lag of development of ligands for this receptor, by overcoming some of these limitations, the commercial development of ligands for the A_{2B} receptor has begun to increase [65].

Asc-397—MRS 1754

MRS 1754 is an adenosine analogue and selective A_{2B} receptor antagonist (K_i =1.97 nM) [22] which has been extremely advantageous in defining the A_{2B} receptor. MRS1754 has reinforced the important role of adenosine in suppressing the inflammatory response and has indicated that A_{2B} receptors are essential in these anti-inflammatory actions [52, 65]. As with many other A_{2B} receptor antagonists, MRS1754 has again reinforced the suggestion that targeting the A_{2B} receptor may prove therapeutically useful [23].

Asc-438—IB-MECA

IB-MECA is a highly potent and selective agonist for the A_3 receptor (K_i =1.1 nM) [22]. The A_3 receptor has widely been implicated as a key receptor in cell growth and proliferation in addition to ischemic precondition [14, 66]. By activating A_3 receptors, IB-MECA suppresses the formation of cAMP and its downstream effectors and as such, has been shown to inhibit the growth of malignant cells [67]. Interestingly, by selectively blocking the A_3 receptor, IB-MECA has also been shown to offer cardioprotection [66]. Further commercial development of A_3 receptor ligands may prove therapeutically useful.

Other ligands that have furthered understanding of purinergic signalling

ATP is co-released with other neurotransmitters including γ -aminobutyric acid, noradrenaline, dopamine, 5-hydroxytryptamine, acetylcholine and glutamate [1].

Glutamate, an amino acid, is the major excitatory neurotransmitter in the mammalian central nervous system and acts at both ionotropic and metabotropic receptors. As such, glutamate is inextricably linked with excitatory synaptic transmission and synaptic plasticity [68]. As the most abundant neurotransmitter and in addition to the widespread functionality glutamate displays, glutamate has also been implicated in numerous physiological and pathophysiological

ological processes, in a similar way to ATP. The co-release of ATP and glutamate is particularly notable and has related purinergic signalling to a number of physiological areas in which glutamate is particularly important.

Synaptic plasticity

A fascinating area in which the purinergic receptors have recently been implicated is synaptic plasticity. The P2X receptors have been shown to play an essential role in synaptic plasticity and display the ability to modulate synaptic plasticity [69]. The P2X receptors are also thought to be involved in long-term potentiation (LTP) which is thought likely to be one of the underlying processes essential to synaptic plasticity and consequently of learning and memory [70]. P2X receptors are also known to interact with other neurotransmitter receptors including the glutamate sensitive *N*-methyl-D-aspartate (NMDA) receptors. NMDA receptors are essential in controlling both synaptic plasticity and memory function and as such, LTP is considered an NMDA receptor dependent process [69].

It is thought that ATP, by acting at P2 receptors affects calcium signalling and subsequently plays an important role in LTP [70]. Indeed, it has been suggested that ATP when co-released with glutamate may activate hippocampal neurons thus allowing calcium to enter postsynaptic cells which consequently inhibits the effectiveness of NMDA receptors to induce LTP. By blocking glutamate receptors with potent, selective 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA)/kainate receptor antagonists NBQX and D-AP5, Pankratov et al. [70] utilised these key receptor ligands in order to suggest that P2X receptors control the activity of NMDA receptors so that weak stimuli do not induce LTP.

Purinergic signalling has additionally been associated with other related areas of synaptic plasticity such as astrocyte function. Until recently, it has generally been considered that astrocytes were ancillary cells of the nervous system. However, recent research has revealed that astrocytes maintain numerous, essential roles in the nervous system. As part of a gliovascular network, astrocyte function includes the maintenance of ion and pH homeostasis, glucose delivery and clearance of neuronal waste such as neurotransmitters released during synaptic transmission [71].

Notably, astrocytes have been shown to express P2Y₁, P2Y₂, P2Y₄ and P2X₇ receptors and by release of so called gliotransmitters, astrocytes can modulate synaptic activity by affecting neighbouring neurons. It is also thought that astrocytes participate in brain intracellular signalling by releasing glutamate. This glutamate release is believed to occur by a number of mechanisms and although these mechanisms are not yet fully understood, it is thought that

the P2X₇ receptor, which is expressed on many cells in the nervous system, may mediate one of these mechanisms [72, 73]. It is further thought that the P2X₇ receptor and potentially other P2 receptors, once activated by ATP, stimulate glutamate release by evoking intracellular Ca²⁺ increase. This in turn is thought to induce glutamate release from astrocytes, allowing subsequent interaction with neighbouring neurons and consequent modulation of synaptic activity [74, 75].

Key glutamate ligands have proved extremely useful in further elucidating this ATP and purinergic receptor-mediated glutamate release mechanism. Fellin et al. [71] antagonised the P2X₇ receptor with Bz-ATP and blocked glutamate receptors with a number of glutamate receptor ligands including D-AP5 (NMDA antagonist), NBQX (AMPA/kainate antagonist), LY367385 (mGlu_{1a} antagonist) and MPEP (mGlu₅ antagonist). This led to the suggestion that the purinergic receptors mediate two glutamate release pathways in astrocytes.

In addition to astrocytes, purinergic signalling has also been implicated in other glial cell processes. For instance, Wurm et al. [72] suggested that a glutamate-purinergic signalling cascade which results in the activation of purinergic P2Y₁ and P1 receptors, results in regulation of glial cell volume which may be important in homeostasis during neuronal activation.

This exciting new area of purinergic signalling research highlights the important role of ATP in an area which, in addition to furthering our understanding of purinergic signalling, emphasises the important role of glial cells and the close relationship between glutamate and ATP. It is clear that the diverse and highly varied role of ATP and purinergic signalling has great impact on pathophysiological research and by further understanding the close relationship between ATP and glutamate, understanding of pathophysiological areas in which glutamate has been implicated may also be furthered.

Glutamate activates ionotropic AMPA, kainate, NMDA and metabotropic receptors and is thought to be involved in many pathophysiologicals. For instance, AMPA, kainate and NMDA glutamate receptors, which have been shown to be expressed in oligodendrocyte lineages, are thought to be related to a number of pathophysiological interactions. In particular, sustained activation of AMPA, kainate and NMDA receptors can lead to cytoplasmic Ca²⁺ overload and is known to damage oligodendrocytes, which in turn can lead to disease [76]. As this oligodendrocyte damage is related to Ca²⁺ levels, the P2X₇ receptor has again been shown to be closely related to this oligodendrocyte damage. Matute [76] recently revealed that P2X₇ receptor expression is elevated in multiple sclerosis (MS) patients, leading to the suggestion that signalling through P2X₇ receptors may be enhanced in MS. This re-emphasises the important

interactions between ATP and glutamate and indeed further research into this area may allow the development of novel therapeutic treatments for some CNS disorders.

Conclusions

It is clear that the purinergic signalling field, whilst previously in its infancy is now in the process of rapidly growing and expanding. Due to the great many physiological and perhaps more important pathophysiological implications of the purinergic signalling field, the requirement for purinergic receptor ligands has and will continue to greatly increase. In order to allow the continued progression and expansion of the purinergic signalling field, it is vital that companies such as Ascent Scientific continue to commercially develop ligands to keep pace with the rapid rate of research and requirement for receptor ligands. This will ensure the continued progress and expansion of the exciting purinergic signalling field.

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